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Clinical Exome Sequencing Identifies a Novel *TUBB4A* Mutation in a Child with Static Hypomyelinating Leukodystrophy

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Abstract

Background—Leukodystrophies are a large group of inherited diseases of CNS myelin. There are few treatments, and a majority of patients do not receive a final genetic diagnosis.

Patient—We report a novel presentation of a female child with hypotonia, global developmental delay, and rotatory nystagmus. Brain MRI demonstrated profound hypomyelination; and minimal or no atrophy in the brain stem or cerebellum.

Results—Extensive testing failed to yield a diagnosis until clinical whole exome sequencing revealed a novel pathogenic mutation in the β-tubulin gene *TUBB4A. TUBB4A* is a cause of hereditary dystonia type 4 (DYT4) and has recently been reported to cause hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC).

Conclusions—This report expands the phenotypic spectrum of *TUBB4A*-associated neurological diseases to include static hypomyelinating leukodystrophy and supports the clinical relevance of next generation sequencing diagnosis approaches.

Keywords

Leukodystrophy; TUBB4A; whole exome sequencing; hypomyelination

Introduction

Leukodystrophies are a group of chronic, inherited disorders that affect development or maintenance of central nervous system (CNS) myelin (Bielschowsky, 1928; Kaye 2001; Maria et al., 2003). Leukodystrophies have an incidence of almost 1 in 7500 live births, but less than half of patients receive a specific genetic diagnosis (Bonkowsky et al., 2010).

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Testing strategies have been proposed based on imaging features on MRI (Schiffman and van der Knaap, 2009) or prevalence (Bonkowsky et al., 2010). However, high costs of single genetic tests, combined with the likelihood that many causes of leukodystrophy have not yet been discovered, continue to limit the ability to make diagnoses.

Steady advances in next generation sequencing technologies are making whole exome sequencing a first-tier option for diagnosis of complex genetic disorders with reported yields of 25% (Yang, 2013). Unbiased genome-wide approaches exemplified by whole exome or whole genome sequencing provide the potential for diagnosis of known diseases without step-wise ordering of multiple individual tests. Further, genomic-wide sequencing can contribute to ongoing discovery of novel disease genes.

We report a 5 year-old female with non-progressive hypotonia and developmental delay, and hypomyelination of the dorsal brainstem, cerebellum, and corpus callosum. After an extensive diagnostic odyssey, whole exome sequencing revealed a mutation in the β-tubulin 4a gene, *TUBB4a*. Mutations in *TUBB4a* have been reported as a cause of hereditary dystonia DYT4 (Hersheson et al., 2012; Lohmann et al., 2012) and more recently as a cause of hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) (Simons et al., 2013). Our report expands the clinical spectrum of *TUBB4A*-associated neurological disease to include static hypomyelinating leukodystrophy.

Case Report

A 5-month-old Caucasian female was referred for pediatric neurology evaluation because of hypotonia and abnormal eye movements first noted at age 2 months. Physical and neurological examination was significant for rotatory nystagmus, hypotonia, and inability to roll over or lift head from a prone position.

Past medical history was significant for a delivery complicated by meconium aspiration and nuchal cord. The patient was diagnosed at ten weeks of age with gastroesophageal reflex, for which she was treated with lansoprazole and ranitidine. There were no family members with leukodystrophies or dystonias; there was no history of consanguinity.

Brain magnetic resonance imaging (MRI) and MR spectroscopy of the brain at age 5 months showed hypomyelination of the dorsal midbrain, cerebellum, and corpus callosum (Figure 1a-c). Repeat brain MRIs at ages 14 months and 33 months had similar findings, although minor atrophy of the brain stem and cerebellum was apparent (Figure 1d-f). Extensive laboratory yielded normal results (Table 1). Formal opthalmological exam nystagmus revealed rotatory nystagmus in all directions of gaze, but normal optic nerves and retina. The nystagmus resolved by age 1 year.

The patient has shown slow attainment of some milestones: at 10 months she was unable to sit up or roll over, but could hold object in her hands, and make babbling vocalizations. At age 2 years she was more reactive to auditory and visual stimuli. When most recently seen at age 4 years she had not achieved other developmental milestones and remains unable to sit unassisted; and had not developed any spasticity, dystonia, or choreoathetoid movements.

Other medical issues that the patient has had include corrective eye surgery for strabismus at age 6 months; placement of a percutaneous gastrostomy tube at age 16 months because of difficulty swallowing solid foods and poor weight gain; and episodes of breath-holding at two years of age for which she was treated with iron supplementation because of slightly depressed serum iron levels.

Clinical whole exome sequencing (Ambry Laboratories) on the patient and both parents revealed a pathogenic missense mutation at exon 4 in the β-tubulin 4A gene (TUBB4A), c. 467G>T (p.R156L). Exome sequencing consisted of full exome sequencing with bioinformatics analysis and filtering based on autosomal and X-linked dominant, or recessive models of inheritance. Manual review to rule out sequencing artifacts and polymorphisms, and to rule out genes lacking clinical overlap, was performed. Two candidate gene alterations were identified, one of which co-segregated with the father and was thus excluded. The *TUBB4A* mutation was not present in either parent.

Discussion

This report describes a female child with hypotonia and global developmental delay with slow attainment of some developmental milestones. By age 5 months hypotonia and rotatory nystagmus were noticed, and a brain MRI showed hypomyelination. Despite an extensive, prolonged, and costly laboratory evaluation, the diagnosis remained unknown until clinical whole exome sequencing was performed revealed a novel pathogenic mutation, p.R156L, in the β-tubulin gene *TUBB4A*.

The *TUBB4A* gene is a β-tubulin highly expressed in the CNS that forms heterodimers with α-tubulin to make cytoskeletal microtubules. Including our case, mutations in TUBB4a have now been reported to cause three neurological diseases: hereditary dystonia type 4 (DYT4) (Hersheson et al., 2012; Lohmann et al., 2012); and hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) (Simons et al., 2013). To date, the mutations in *TUBB4A* known to cause dystonia are p.R2G and p.A271T, while H-ABC is caused by D249N. In our patient, mutation of the amino acid R156 is likely pathogenic because of the non-synonymous amino acid change (from arginine to leucine); and because arginine at this location in the protein is highly conserved through evolution, including in vertebrates chimpanzee, mouse, zebrafish; and even in the invertebrate flatworm *Caenorhabditis elegans*. Crystallography has demonstrated that R156 is in the conserved α-helix 4 of TUBB4A (Lowe et al., 2001).

Patients with H-ABC were initially grouped by their MRI imaging patterns that showed atrophy in cerebellar white matter, neostriatum, and cerebellum (Schiffman and van der Knaap, 2009). In 2013 Simons et al. performed whole exome sequencing in families with H-ABC and discovered a causative pathogenic variant in *TUBB4A*. Some shared clinical features between the patient reported here and patients with H-ABC include the presence of nystagmus, sometimes rotatory; developmental delay, particularly motor; and hypotonia. However, our report expands the phenotypic spectrum of *TUBB4A* gene mutations to include static hypomyelinating leukodystrophy without basal ganglia atrophy. Also, the cerebellar and brain stem atrophy by age 33 months is minimal. Other features that differed

in the patient reported here were a more severe early disease course with onset of symptoms in the first months of life; failure to obtain developmental milestones including sitting, walking, or talking; an absence of extrapyramidal and cerebellar symptoms; and absence of clinical deterioration. The patient reported here did not have dystonia or dysphonia, in contrast to patients with DYT4-associated TUBB4A mutations (Hersheson et al., 2012).

This case illustrates an example where whole exome sequencing was used to end a diagnostic odyssey in a patient where extensive genetic heterogeneity would have made step-wise genetic diagnosis logistically difficult and prohibitively expensive. Next-generation sequencing technologies provide the potential for unbiased diagnosis of known diseases without individual ordering of multiple individual tests, and will contribute to discovery of novel disease genes. However, continued limitations of this technology include substantial cost up to $15,000-$20,000, although these numbers are rapidly decreasing; potential for identifying unanticipated disease variants unrelated to the test indication; potential false negatives due to imperfect exome coverage; and methodological limitations in the interpretation phase if a clear disease-associated variant is not identified. This last problem is significant due to the large number of deleterious gene variants in all humans that could plausibly be related to a phenotype (especially in the CNS), which could yield false-positive associations.

Whole exome sequencing continues to become more accessible, and may become the method of choice for the diagnosis of leukodystrophies. A rational, cost-effective algorithm for leukodystrophy diagnosis could be developed, with a tiered approach to testing. For example, first tier testing could include for treatable leukodystrophies, for leukocyte lysosomal enzyme testing, and in males, for very long chain fatty acids. The second tier could be whole exome sequencing. However, without sufficient data on the sensitivity of whole exome sequencing for leukodystrophies, and on the false negative rate for missing gene deletions or duplications (such as for Pelizaues-Merzbacher or vanishing white matter disease) this approach is not yet indicated.

Our findings underscore the importance of TUBB4A in the development of myelin in the CNS. The biochemical role of TUBB4A in myelin development and maintenance is not understood; for example, whether TUBB4A is required for formation of the myelin sheath. It has been proposed that TUBB4A might primarily play a role in neuron development, and that the hypomyelination might be a secondary phenotype (van der Knaap et al., 2007; Simons et al., 2013). Currently however there is no evidence to support or refute the precise role(s) of TUBB4A.

TUBB4A-associated mutations have now been demonstrated to be a cause of hypomyelinating leukodystrophies, and our case demonstrates that the phenotypic spectrum is broader than initially described. Certain diagnostic features such as cerebellar and brain stem atrophy may not present until an older age, complicating recognition and diagnosis (Simons et al., 2013). While there is currently no cure for patients with TUBB4A-associated leukodystrophy, symptomatic treatment is possible, for example if dystonia is present (Simons et al., 2013). Further, there is utility in making an expedient diagnosis by excluding

treatable forms of leukodystrophies; curtailing other expensive and lengthy testing; and providing valuable reassurance and prognostic information to the patient and their family.

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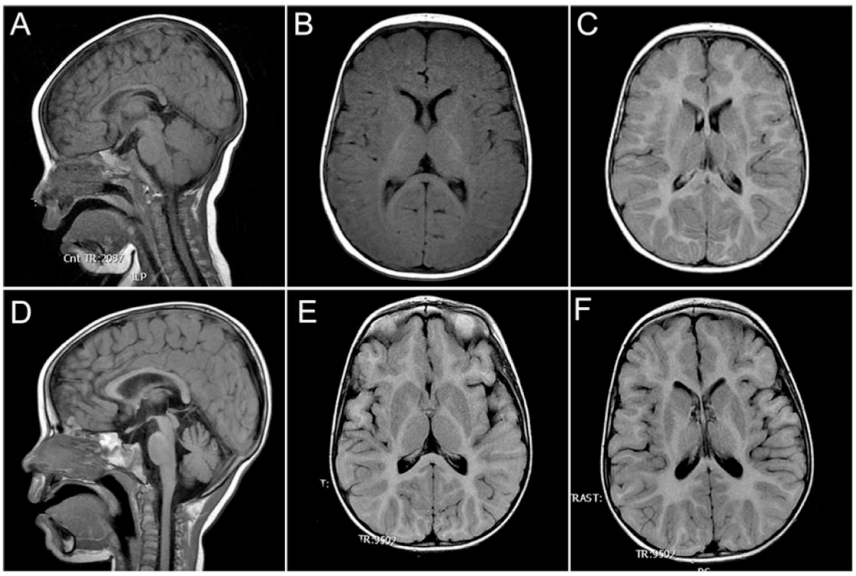

Figure 1.

Brain MRI images; A-C; at age 5 months; D-F at age 33 months. A) Sagittal T1 shows hypomyelination but no atrophy of the brainstem or cerebellum. B) Axial T1 shows diffuse hypomyelination. C) Axial T2 FLAIR demonstrates normal size of basal ganglia. D) Sagittal T1 image shows persistent hypomyelination but minimal atrophy of the pons and cerebellum. E-F) Axial T2 FLAIR images (two different cuts; Axial T1 imaging was not performed) show absence of basal ganglia atrophy with normal size of putamen.

Table 1

List of laboratory testing performed prior to whole exome sequencing; all results were normal.

Chemistry 10 panel
Complete blood count
Liver function testing (AST, ALT, GGT, Albumin, Bilirubin)
Thyroid testing (TSH, FT4)
serum amino acids
urine organic acids
urine homovanillic acid
urine vanillymandelic acid
leukocyte lysosomal enzymes
urine sialic acid
transferrin isoelectric focusing
comparative genomic hybridization microarray
GLC2 gene sequencing
PLP1 gene sequencing